

Chemical composition and physical parameters of particles as factors of variability of the sorption properties of protein powder preparations

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Abstract. Plant powders with high protein concentration are of growing interest to consumers. The aim of the study was to identify the relationship between sorption properties and the chemical composition and physical parameters of high-protein powders from pumpkin, pea, sunflower, rice, and hemp. Sorption properties were estimated using Brunauer, Emmet, and Teller; Guggenheim, Anderson, and de Boer; and Peleg models based on data obtained with the static-desiccator method. The chemical composition was examined using chemical methods, while the physical parameters were determined using the Morphologi GS automatic structure analyzer. A synthetic evaluation of the correlation between sorption properties and the chemical composition and physical parameters of powder particles was carried out based on the comparative analysis of multiple regression equations. It was found that the sorption properties of high-protein plant powders were mainly determined by their chemical composition, in which the share of fiber turned out to be particularly important. Among the physical parameters, the diameter and circularity of particles turned out to be important.

Keywords: sorption properties, sorption models, microbiological stability, plant powders, plant protein preparations, physical parameters of particles

1. INTRODUCTION

Many new plant-based products in the form of powder appearing on the food market are a rich source of protein. Protein determines the nutritional value of food and influences its physical structure through such processes as

solubilization in water, water and fat binding, emulsification, foaming, gelling, and dough formation. Therefore, the physical properties of protein-rich particles shape the sensory quality of food. These properties are determined by interactions of various protein fractions with other organic or inorganic substances and by the size of their molecules, their structure (degree of substitution), and charge distribution in molecules. They are also strongly affected by the environment in which proteins occur during food processing (Day, 2013).

There is a common view that still much needs to be elucidated regarding the functional properties of proteins linked with their specific physical nature. This is strongly associated with their structural properties revealed in certain conditions. Proteins can be used to create fibrillar structures that mimic meat (Xiaonan *et al.*, 2024) or soft gel-like structures that mimic whey proteins, which can be applied as fat substitutes in diets (Day, 2013). Therefore, extending knowledge about the properties of novel food products seems essential, especially that the greater demand for plant proteins in food envisaged in the future will be associated with the development of their novel sourcing technologies.

High-protein rice products are by-products of rice starch processing from rice bran and broken rice grains (Fabian and Ju, 2011). They have been increasingly used to



produce gluten-free foods owing to the hypoallergenicity of rice protein (Shih, 2003). This protein is, however, not widely available on the market.

Pea flour is produced by dry milling of dehulled peas. Pea protein concentrate is obtained via dry separation and pea protein isolate via wet processing using solubilization with a base or an acid and isoelectric precipitation or ultra-filtration (Boye *et al.*, 2010). The growing popularity of pea proteins stems from their capability to bind lipids and water, their emulsifying and gelling properties, as well as their texture and nutritional value (Sandberg, 2011).

Sunflower meal is rich in crude protein, however, the usability of sunflower proteins depends on the oil extraction method. A powder obtained with solvent extraction or high-temperature treatment has high content of insoluble proteins. Therefore, sunflower proteins are primarily used as animal feedstuff (Gonzalez-Perez and Vereijken, 2007).

Pumpkin seed meal is also dense in protein (60-65%) with low contents of anti-nutrients. Therefore, protein preparations from pumpkin seeds may be used to produce foods with nutritional and health-promoting properties (Vinayashree and Vasu, 2021).

Hemp seed meal is an excellent alternative source of plant protein, from which fractions rich in protein and fiber can be separated by dry grinding and sieving (Pojić *et al.*, 2014; Hadnađev *et al.*, 2018). Hemp seed protein isolates lack anti-nutrients and contain biologically active compounds (Mamone *et al.*, 2019).

High-protein plant-derived powders, representing materials with strongly developed surface and sorption properties, are susceptible to changes triggered by their interactions with water vapor that will determine their microbiological safety and stability (Fu *et al.*, 2023). It should be remembered that such plant preparations are not sterile. Therefore, in conditions of increased humidity, their chemical composition and microbiological quality and, consequently, also safety may deteriorate significantly.

The basic tool applied to analyze various quality parameters of food powders is sorption isotherms. They illustrate the specific amount of water retained (*i.e.*, the water holding capacity) by solid food ingredients as a function of

water activity (a_w), which is determined at a stable temperature. Water vapor sorption by food depends on its chemical composition, physiochemical state of its ingredients, and physical structure, including porosity. Various reactions, including those being of key importance to the food quality and safety like increasing microbial counts and changes in the physical state, follow well known patterns linked with the specified a_w level (Roudaut, 2020). Hence, the water vapor sorption isotherm is an extremely valuable tool in predicting which reactions will diminish stability at the specified moisture content, it aids the choice of ingredients aimed to modify a_w and increase stability and may be used to predict moisture content increase or loss in a package with known moisture permeability (Labuza and Altunakar, 2020). The present study was aimed at comparing the sorption properties of five protein powder preparations using three sorption models and at establishing correlations between the chemical composition and physical parameters of their particles and the variability of their sorption properties.

2. MATERIALS AND METHODS

2.1. Experimental material

The research material consisted of 5 protein preparations (made from pumpkin seeds, peas, sunflower seeds, rice, hemp) in the form of powder produced in industrial conditions for GK4 Food Sp. z o.o. seated in Trzebiatów (Poland). They were intended for consumers looking for high-protein plant-derived preparations that are easy to prepare for consumption and were purchased at retail. The individual protein powder preparations differed not only in their sensory properties (color, aroma, taste) but above all in their chemical composition, which is presented in Table 1 based on the results of our research.

2.2. Methods

The chemical composition of the tested powders was determined based on the content of water in accordance with PN-A-79011-8:1998, protein in accordance with PB-116 ed. III 11.08.2020, carbohydrates in accordance with

Table 1. Chemical composition of the tested protein powder samples

Component (g 100 g ⁻¹)	Protein source				
	Pumpkin	Pea	Sunflower	Rice	Hemp
Water	7.4±0.1 ^b	7.9±0.1 ^c	9.4±0.2 ^d	6.8±0.2 ^a	8.2±0.2 ^c
Protein	60.6±1.8 ^b	80.7±2.4 ^c	46.4±2.3 ^a	89.9±2.7 ^d	48.2±2.4 ^a
Lipids	15.1±1.4 ^c	8.1±0.7 ^b	12.8±1.2 ^c	6.7±0.6 ^a	9.1±0.8 ^b
Carbohydrates	2.4	0.7	6.8	0.3	3.3
Fiber	12.7±2.5 ^b	4.9±1.2 ^a	19.2±3.8 ^c	3.9±1.0 ^a	26.7±5.3 ^c
Starch	<0.2 ^a	0.7±0.2 ^c	<0.2 ^a	0.3±0.1 ^b	<0.2 ^a
Ash	7.32±0.44 ^c	4.24±0.25 ^b	6.74±0.40 ^c	0.57±0.03 ^a	8.63±0.52 ^d

Source: results of own research.

Regulation (EU) No 1169/2011 of the European Parliament, dietary fiber in accordance with AOAC 991.43:1994, starch in accordance with PB-265 ed. II 27.11.2020, and minerals in accordance with PN-A-79011-8:1998.

Selected physical parameters (diameter, circularity, convexity, elongation, shape coefficient, and solidity) characterizing the size and shape of particles were determined using the Morphologi G3 automatic particle analyzer (Malvern Instruments, United Kingdom), which enables examining the distribution of parameter values of solid particles with sizes ranging from 0.5 to 10,000 μm .

Water activity was determined based on the dew point in the AquaLab apparatus (Series 4 model TE by Decagon Devices, Inc., Pullman, WA, USA) with an accuracy of ± 0.003 at a temperature of 293.15 K (Ociecek *et al.*, 2022).

Sorption isotherms were determined using the standard static-desiccator method. The water content and water activity of the samples were determined in the initial state and in the state of equilibrium in the atmosphere having a specific relative humidity, which was regulated using saturated solutions of appropriate substances. Analyses were conducted in the water activity range from 0.07 to 0.82 and at a temperature of 293.15 K (20°C). The time needed for the system to reach the equilibrium state was 8 days from placing the samples in the desiccators. In the analytical variant with $a_w \geq 0.7$, thymol was placed in the desiccators to protect the samples against microflora development. All protein powder samples (approx. 1 g \pm 0.1 mg) intended for determinations of sorption isotherms in the first stage of the experiment were placed in measuring vessels and, in the next stage, the vessels were placed in a desiccator containing P_2O_5 (a dehydrating agent) at room temperature for 7 days in order to reduce the moisture content of the samples (~2%). After 7 days, the samples were weighed and placed in desiccators with saturated solutions of the appropriate substances. The equilibrium water content was calculated and adsorption isotherms were plotted using the MS Excel program based on the initial mass of the product (determined after 7-day incubation in a desiccator with P_2O_5) and changes in the water content. The water activity of the samples was measured using the AquaLab apparatus 8 days after placing them in the desiccators. Adsorption isotherms were plotted using the Excel 2013 program (Ociecek *et al.*, 2022).

The mathematical description of the empirically determined sorption isotherms was made using three mathematical models of sorption isotherms differing in the number of parameters, including two theoretical models by Brunauer, Emmet, and Teller (BET – a two-parameter model) and Guggenheim, Anderson, and de Boer (GAB – a three-parameter model) and one empirical model (a four-parameter Peleg model). The choice of the models was not accidental, as it was expected to enable the assessment of the efficiency of models with various levels of complexity in describing data sets characterizing high-protein materi-

als. Their choice was also driven by the need to estimate physical parameters specific to the sorption process and to estimate model parameters which are of no physical significance but can be compared and serve to differentiate the analyzed samples.

BET equation (Eq. (1)) (Figura and Teixeira, 2007; Pałacha and Sitkiewicz, 2010):

$$v = \frac{v_m C_{BET} a_w}{(1-a_w)[1+(C-1)a_w]}, \quad (1)$$

where: a_w – water activity (–), v – equilibrium water content (g H_2O 100 g d.m.⁻¹), v_m – water content in the monolayer (g H_2O 100 g d.m.⁻¹), C_{BET} – energy constant (kJ mol⁻¹).

GAB equation (Eq. (2)) (Figura and Teixeira, 2007; Pałacha and Sitkiewicz, 2010):

$$v = \frac{v_m C_{GAB} K a_w}{(1-K a_w)(1-K a_w + C_{GAB} K a_w)}, \quad (2)$$

where: C_{GAB} – Guggenheim energy constant (kJ mol⁻¹), K – constant correcting properties of multilayer molecules compared to the liquid phase.

Peleg equation (Eq. (3)) (Andrade *et al.*, 2011):

$$v = A a_w^B + D a_w^E, \quad (3)$$

where: A – constant (–), B – constant (–), D – constant (–), E – constant (–).

Knowing the volume of water vapor adsorbed at a temperature lower than the boiling point and the so-called water setting surface, the specific surface area of the adsorbent was computed based on Eq. (4) (Paderewski, 1999):

$$a_{sp} = \omega \frac{v_m}{M} N, \quad (4)$$

where: a_{sp} – specific sorption area (m² g⁻¹), N – Avogadro number (6.023 $\times 10^{23}$ molecules mol⁻¹), M – molecular weight of water (18 g mol⁻¹), ω – water cross-section area (1.05 $\times 10^{-19}$ m² molecule⁻¹).

The sizes and volumes of the capillaries of the analyzed powders were determined for the capillary condensation area using the Kelvin equation (Eq. (5)) (Paderewski, 1999) and assuming the cylindrical shape of the capillaries (Figura and Teixeira, 2007):

$$\ln a_w = \frac{2\sigma V}{r_k R T}, \quad (5)$$

where: σ – surface tension of the liquid at temperature T (N m⁻¹), r_k – capillary radius (nm), R – universal gas constant (kJ mol⁻¹ K⁻¹), T – process temperature (K), V – molar volume of the adsorbate (m³ mol⁻¹).

2.3. Methods of statistical analysis

The chemical composition and water activity of the tested protein powder samples were expressed as an arithmetic mean with standard deviation estimated from three parallel determinations. The statistical significance of differences between means was assessed using the ANOVA test and Tukey's post-hoc test (Łomnicki, 2006).

Differences in the course of the sorption isotherms in the entire a_w range were analyzed statistically using the Student's t-test for bonded pairs and were considered statistically significant at a significance level of $\alpha = 0.05$ (Łomnicki, 2006).

Parameters of all equations were estimated based on empirical data using non-linear regression and a Monte Carlo algorithm, which enabled avoiding the arrestment of the estimation process by the local minimum. Calculations were performed in the Excel 2013 calculating sheet using a the Solver macro-command. Errors in parameters determined for particular equations were estimated with the SolverAid macro-command. The BET model parameters were estimated for the a_w range from 0.05 to 0.35, whereas those of the GAB and Peleg models – for the entire a_w range tested (Andrade *et al.*, 2011).

The fit of empirical data to the equations was characterized based on the evaluation of the root sum squared (RSS) (Eq. (6)):

$$RSS = \sum(v_e - v_0)^2, \quad (6)$$

and the value of the root mean square error (RMSE) (Eq. (7)) expressed in %:

$$RMSE = \sqrt{\frac{\sum\left(\frac{v_e - v_0}{v_e}\right)^2}{N}} 100\%, \quad (7)$$

where: N – number of data, v_e – experimental equilibrium water content (g H₂O 100 g⁻¹ d.m.), v_0 – equilibrium water content predicted with the model (g H₂O 100 g⁻¹ d.m.) as the criteria most frequently used in statistical analysis (Basu *et al.*, 2006; Ocieczek *et al.*, 2022; Pałacha and Sas, 2016).

The correlations between the chemical composition and physical parameters of the particles of the analyzed powders and the variability of their sorption properties were examined using multiple regression equations. In these equations, the monolayer capacity was the dependent variable, whereas chemical parameters, such as the content of protein, fiber, and lipids, and physical parameters of the powders, such as diameter, circularity, elongation, and shape coefficient, served the role of independent variables. Due to the access to only five data sets used to estimate the parameters of multiple regression equations, equations with three independent variables were determined. In addition, statistics, such as multiple R², standard error of estimation and F values, were estimated, which served to assess the predictions of the dependent variable by the tested combinations of dependent variables. This assessment was made using the REGLINP() function (Carlberg, 2012).

3. RESULTS AND DISCUSSION

It was shown more than 30 years ago that the physical parameters of particles constituting a mixture are of great importance during its interaction with water molecules, as they influence the diffusivity of water and determine both

its shelf life and quality (Marousis *et al.*, 1991). However, the results of the research conducted by Murrieta-Pazos *et al.* (2014) additionally showed that, at high relative humidity, the protein matrix changes its structure, allowing the formation of bridges, causing agglomeration. Small-sized particles are more susceptible to agglomeration. Furthermore, small particles show greater water adsorption and lower water diffusion coefficients, which can be explained by the specific surface which is inversely proportional to the particle size (small particles have a larger specific surface). Hence, both the storage stability and usability of powders are a function of the physical properties of their particles. Therefore, the major parameters of particles that enable their characterization and differentiation are their physical parameters describing their size as a diameter and their shape through circularity, elongation, convexity, solidity, and shape coefficient (Table 2).

A comparative analysis of particle diameters of the analyzed powders allowed concluding that they differed significantly in this respect. The pumpkin seed powder had the largest particles (18.8 μm), whereas the particles of the rice powder were the finest (8.6 μm). The diameters of particles of the other analyzed powders were similar (13.7 – 16.2 μm). The particle diameters of the analyzed powders were less diversified than those of maize starch modified with various methods, ranging from 11.09 to 14.18 μm (Ocieczek *et al.*, 2017). This comparison indicates that even powders with the same chemical composition can differ significantly in terms of physical parameters. The physical properties of particles are also influenced on rehydration of powders. Wettability, *i.e.* the time it takes a given quantity of powder to sink below the surface of water at a certain temperature, improves with increasing particle size (Murphy *et al.*, 2020). Hence, based on the findings reported by Murrieta-Pazos *et al.* (2014), it can be assumed that this knowledge can be used in the future to better control powder hydration and the agglomeration mechanism in both production and storage conditions.

Particle shape is a complex geometric characteristic. It involves the form and habit of the particle (Pabst and Gregorova, 2007). An interesting parameter of the particle shape of the tested powders was their circularity, which determines how close the particle shape is to a perfect circle. A perfect circle has a circularity of 1.0, while a narrow, elongated object has a circularity close to 0. Circularity is a viable measure of deviation from a perfect circle. Analyzing the values of the numerical distribution of circularity, it may be concluded that the pea powder particles had a regular shape, although more similar to a square (0.852±0.168) than to a circle. In turn, the rice powder particles resembled rectangles (0.685±0.185). The shape of particles can affect flowability of powders, as more spherical powders have greater flowability (Murphy *et al.*, 2020). The elongated shape of the particles may also be due to the use of certain technological processes, *e.g.*, modification of

Table 2. Selected physical characteristics of the tested protein powder samples

Characteristics of value distribution	Parameter					
	Min.	Max.	Mean \pm SD	D [n, 0.1]	D [n, 0.5]	D [n, 0.9]
Pumpkin seeds protein (D) (particles counted $n = 140\,881$) (optic used: $10\times$)						
Diameter (μm)	1.09	336.53	18.75 ± 13.48	6.03	16.44	31.87
Circularity	0.028	1.000	0.750 ± 0.151	0.543	0.777	0.913
Elongation	0.000	0.932	0.281 ± 0.143	0.100	0.270	0.474
Convexity	0.330	1.000	0.956 ± 0.054	0.891	0.966	0.993
Solidity	0.096	1.000	0.938 ± 0.064	0.859	0.948	0.987
Shape coefficient	0.068	1.000	0.719 ± 0.143	0.524	0.728	0.898
Pea protein (G) (particles counted $n = 55\,195$) (optic used: $10\times$)						
Diameter (μm)	1.09	189.63	16.21 ± 13.95	4.13	12.04	33.49
Circularity	0.004	1.000	0.852 ± 0.168	0.640	0.918	0.970
Elongation	0.000	0.931	0.177 ± 0.149	0.037	0.133	0.380
Convexity	0.308	1.000	0.975 ± 0.055	0.900	0.989	0.997
Solidity	0.106	1.000	0.970 ± 0.070	0.908	0.986	0.997
Shape coefficient	0.069	1.000	0.823 ± 0.149	0.617	0.865	0.960
Sunflower protein (S) (particles counted $n = 202\,844$) (optic used: $10\times$)						
Diameter (μm)	1.09	255.47	13.74 ± 9.47	4.79	11.97	23.32
Circularity	0.026	1.000	0.736 ± 0.156	0.515	0.770	0.899
Elongation	0.000	0.928	0.324 ± 0.155	0.125	0.315	0.532
Convexity	0.320	1.000	0.961 ± 0.057	0.893	0.975	0.994
Solidity	0.161	1.000	0.942 ± 0.067	0.856	0.956	0.990
Shape coefficient	0.072	1.000	0.676 ± 0.155	0.466	0.683	0.873
Rice protein (R) (particles counted $n = 1\,005\,259$) (optic used: $10\times$)						
Diameter (μm)	1.09	191.41	8.64 ± 7.34	2.10	6.53	17.37
Circularity	0.024	1.000	0.685 ± 0.184	0.412	0.719	0.894
Elongation	0.000	0.921	0.366 ± 0.162	0.150	0.362	0.582
Convexity	0.290	1.000	0.956 ± 0.068	0.848	0.972	0.995
Solidity	0.145	1.000	0.930 ± 0.090	0.780	0.944	0.990
Shape coefficient	0.079	1.000	0.634 ± 0.162	0.415	0.635	0.847
Hemp protein (K) (particles counted $n = 210\,219$) (optic used: $10\times$)						
Diameter (μm)	1.09	273.41	15.42 ± 12.15	4.84	12.71	27.46
Circularity	0.031	1.000	0.722 ± 0.160	0.488	0.757	0.891
Elongation	0.000	0.936	0.328 ± 0.152	0.130	0.321	0.530
Convexity	0.305	1.000	0.956 ± 0.059	0.879	0.972	0.994
Solidity	0.179	1.000	0.934 ± 0.073	0.834	0.951	0.988
Shape coefficient	0.064	1.000	0.672 ± 0.152	0.467	0.677	0.868

Source: results of own research. SD – standard deviation, D [n, 0.1] – 10% of the particles are smaller than this diameter, D [n, 0.5] – half of the particles are smaller than this diameter, half are longer than this diameter, D [n, 0.9] – 90% of the particles are smaller than this diameter.

starch, such as pre-gelatinization, when elongation reaches values of up to 0.587-0.551 (Ocieczek *et al.*, 2017). The results obtained in this study correspond to the results describing the shape of particles by their elongation. The rice powder particles had the highest value of elongation (0.366), and the pea particles had the lowest one (0.177). For comparison, the elongation of pregelatinized maize starch particles was reported to range from 0.406 to 0.440 (Ocieczek *et al.*, 2017). The results of a study conducted by Murphy *et al.* (2020) on the quality of different types of milk powders showed that the presence of intact proteins and the absence of large lactose crystals provide particles with high circularity and low elongation. In contrast, intact proteins and large lactose crystals cause the circularity of the particles to become lower and elongation significantly higher.

One of the most important quality features of powdered products is their dispersibility as one of the measures of their susceptibility to rehydration. The dispersibility of milk powder is greater when its particles have a more irregular shape (Ding *et al.*, 2020). Therefore, the next analyzed parameter was convexity, which is a measure of particle surface roughness. Particles with a smooth shape have a convexity equal to 1, while the convexity of an irregular, “spiky” object approximates 0. Hence, the results obtained in the present study indicate that the particles of all the tested powders were characterized by smoothness and high uniformity (0.956-0.975). Similar convexity values were reported for modified maize starch particles, which were also indistinguishable in terms of this parameter (Ocieczek *et al.*, 2017). The shape of particles is also described by solidity, which expresses the ratio of the actual surface of the object to the surface formed by the thread stretched around the particle. The comparison of the values of this parameter indicates that the particles of all tested powders did not differ significantly from each other. Convexity and solidity were the parameters that did not allow distinguishing between the tested samples. The results of tests of instant whole milk powder showed that the convexity and solidity of particles are important parameters of its usability (Ding *et al.*, 2020).

The last parameter analyzed was the particle shape coefficient, which is a ratio of the maximum to the minimum Feret diameter of the particle (also often used for the ratio of length to breadth for fairly short particles, when breadth is nearly equal to thickness) (Merkus, 2009). The particle shape coefficient reached the highest value in the case of the pea powder particles (0.823) and the lowest in the case of the rice powder particles (0.634). These differences can be considered significant, but those observed between the particles of the high-protein powders based on rice, hemp, and sunflower seeds were insignificant.

Based on data above, it may be concluded that the tested powders differed significantly in their physical properties, including their diameters, circularity, elongation, and shape coefficients. In contrast, convexity and solidity were the parameters that did not differentiate their particles.

Equally important characteristics of high-protein plant preparations that determine their sorption capacity are their water content and their water activity (Table 3). The analyzed powders differed significantly in terms of both water content ($p=0.0002 \div 0.0132$) and water activity ($p=0.0002 \div 0.0009$). No significant differences in terms of water content ($p=0.0843$) or water activity ($p=0.9901$) were found only between the pea and hemp powders. The highest water activity value, reflecting the thermodynamic state of water, was identified in the sunflower powder (0.51), while the lowest in the hemp powder (0.37) (Table 3). At the same time, it should be noted that the water activity in all the tested preparations ensured their storage stability because it was lower than 0.6, which prevents microflora proliferation (Tapia *et al.*, 2020).

Water is an integral component of essentially all food products influencing their functional properties (Lewicki, 2004) and their susceptibility to degradation, including microbiological degradation, which determines not only their quality (Figura and Teixeira, 2007; Pałacha and Sitkiewicz, 2010) but also, above all, their safety (Tapia *et al.*, 2020). It should be emphasized that although water activity is strongly related to its content, it does not depend only on this parameter.

Water activity is determined by both the chemical composition and physical structure of particles coordinating water molecules (Venir and Maltini, 2013), which may differ significantly. The sorption of the analyzed products was largely determined by the relationship (expressed as a quotient) between the content of water and water activity it generates. The analysis of this relationship showed that water molecules entered into strongest reactions with the surface of the particles of the hemp powder (22.35), although the water content in this powder was not the lowest among the others. This is probably related to the high content of a hydrophilic component, *i.e.* fiber (Ocieczek and Makala, 2019). In turn, the water molecules exhibited the weakest affinity to the particles of the pumpkin powder (16.23), although the water content in this powder was not the highest among the others. This is presumably due to

Table 3. Water content and water activity of the tested protein powder samples (n=3)

Protein source	Water content \pm SD (g H ₂ O 100 g ⁻¹ d.m.)	Water activity \pm SD (-)
Pumpkin	7.3550 \pm 0.0898 ^b	0.4533 \pm 0.0045 ^c
Pea	7.8803 \pm 0.0296 ^c	0.3710 \pm 0.0008 ^a
Sunflower	9.4273 \pm 0.1615 ^d	0.5051 \pm 0.0109 ^d
Rice	6.8533 \pm 0.1527 ^a	0.4038 \pm 0.0088 ^b
Hemp	8.2367 \pm 0.2254 ^c	0.3686 \pm 0.0014 ^a

the high content of a hydrophobic component, *i.e.* lipids (Mukherjee, 2018) (Table 1). This relationship, however, is definitely more complex than the mutual ratio of these two components and will therefore be subject to in-depth analysis later in the work.

Sorption isotherms are one of the best tools for both examining the mechanism of surface adsorption and providing the basis for determining such parameters as monolayer capacity, the extent of change in energy accompanying the sorption process or estimating the radii of capillaries filled after initiating capillary condensation, and estimating the total volume of capillaries in the capillary condensation area (Basu *et al.*, 2006). The obtained data (Fig. 1) indicate that the sorption mechanism followed the same pattern in all the tested powders and that the sorption isotherms had a sigmoidal shape (Figura and Teixeira, 2007; Ociecek and Makala, 2019; Ociecek *et al.*, 2022). One of the elements in assessing the similarity of isotherms is the statistical assessment of their course carried out based on

data describing the equilibrium water content in the tested range of a_w values (Fig. 1f). Its results showed a statistically significant difference ($t_{0.05} = 2.228$) between the sorption isotherms of protein of pumpkin and pea ($t_{D/G} = 2.636$), pumpkin and rice ($t_{D/R} = 2.289$), pea and rice ($t_{G/R} = 5.709$), sunflower and rice ($t_{S/R} = 7.210$), and rice and hemp ($t_{R/K} = 9.583$).

In turn, no significant difference ($t_{0.05} = 2.228$) was found between the sorption isotherms of powders from pumpkin and sunflower ($t_{D/S} = 1.950$), pumpkin and hemp ($t_{D/K} = 0.583$), pea and sunflower ($t_{G/S} = 0.156$), pea and hemp ($t_{G/K} = 1.102$), and sunflower and hemp ($t_{S/K} = 1.931$).

Water sorption isotherms are important thermodynamic tools for predicting interactions between water and food constituents (Lewicki, 2004; Figura and Teixeira, 2007). However, due to the fact that statistical comparison of the course of sorption isotherms over the entire a_w range allows identifying differences only between preparations with very different characteristics, the data achieved were

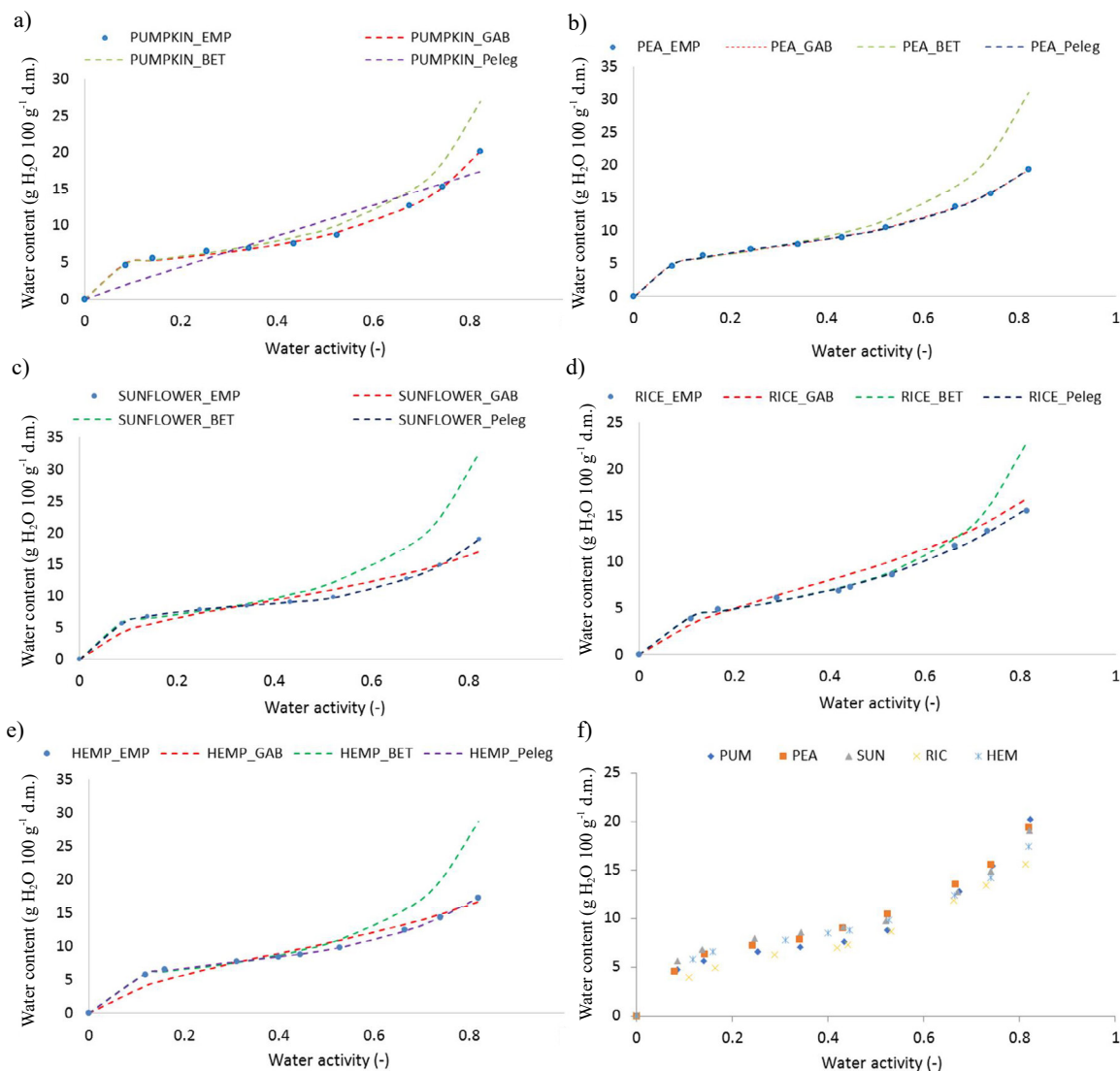


Fig. 1. Sorption isotherms of protein powders: a) pumpkin seed, b) pea, c) sunflower, d) rice, e) hemp, f) all.

explored using three mathematical models. Two theoretical models: BET and GAB (Labuza and Altunakar, 2020) are most frequently used in analyses of food products, which enable estimating parameters that characterize sorption properties, *i.e.*, monolayer capacity and energy constant (Tables 4 and 5).

The BET and GAB models are based on the assumption that the adsorbent contains a large number of independent and equivalent adsorption centers on which multilayer adsorption can develop (Vopička *et al.*, 2022). However, the applicability of the BET model is limited to a relatively narrow a_w range (0.05-0.35) (Figura and Teixeira, 2007; Condon, 2019; Aviara, 2020). At a_w below 0.05 and above 0.35, a significant overestimation of the BET function can be observed compared to the empirical results. However, the low *RSS* and *RMSE* values indicate that the BET model (Pałacha and Sas, 2016; Aviara, 2020) fitted well to the data describing surface water adsorption in this limited range of a_w values. In turn, the relatively low values of the C_{BET} energy constant of this model estimated for most powder samples indicate that the studied phenomenon was of a physical nature (Condon, 2019). The very high C_{BET} value estimated for one of the samples can probably be attributed to the mathematical compensation of its value performed in order to obtain an optimal solution for the remaining parameters (Table 4). Moreover, the estimated monolayer capacity values were within the food-specific range indi-

cated by Karel (1975). This parameter is an indicator of the availability of polar sites for water molecules, regardless of which fraction is the source of hydrophilic groups (Pałacha and Sitkiewicz, 2010).

However, taking into account that the BET model is a special case of the GAB model (Kludský *et al.*, 2018) and that it has certain limitations in describing sorption isotherms, the sorption parameters were estimated also based on the GAB model (Table 5), which is used for a much wider range of a_w data, *i.e.*, $a_w < 0.93$ (Basu *et al.*, 2006; Aviara, 2020).

The fit of the empirical data describing surface water adsorption in a broader range of a_w values (0.00÷0.82) to the GAB model was slightly poorer compared to the BET model. However, the computed *K* values, which fitted within the range of $0.24 < K \leq 1$, allow concluding that the GAB equation was correctly used to describe the experimental data (Lewicki, 1997). The values of the energy constant C_{GAB} confirm that the surface interaction of the tested powders with water molecules was of a physical nature (McMinn *et al.*, 2003; Condon, 2019). In turn, the monolayer capacity values correlated quite well with the previously determined relations (quotients) between the water content and the relative vapor pressure it creates, sometimes considered tantamount to water activity (Reid, 2020). Therefore, it should be noted that the GAB model better reflects the complex relationships between the surface of a solid body and water molecules than the BET model.

Table 4. Parameters of the BET equation for the analyzed protein powder samples

Protein source	BET model parameter		Complementary parameter	Measures of fit	
	<i>C</i> (kJ mol ⁻¹)	v_m (g H ₂ O 100 g ⁻¹ d.m.)	a_w at v_m (-)	<i>RSS</i> (-)	<i>RMSE</i> (%)
Pumpkin	354.50 ± 0.2018	4.7015 ± 1.7400	0.0948	0.7982 ± 0.4829	5.9661
Pea	79.696 ± 0.3469	5.4555 ± 1.5949	0.1188	0.8314 ± 0.7656	7.1056
Sunflower	351.20 ± 0.2983	5.6948 ± 1.9437	0.0997	1.3822 ± 0.7536	6.7481
Rice	45.568 ± 41.242	4.2933 ± 0.2993	0.1345	0.3631 ± 0.6026	5.1555
Hemp	1.55×10 ¹¹ ± 4.55×10 ¹⁹	5.1822 ± 0.1336	0.1084	0.2694 ± 0.5191	4.6583

Source: results of own research.

Table 5. Parameters of the GAB equation for the analyzed protein powder samples

Protein source	GAB model parameter			Complementary parameter	Measures of fit	
	<i>K</i> (-)	<i>C</i> (kJ mol ⁻¹)	v_m (g H ₂ O 100 g ⁻¹ d.m.)	a_w at v_m (-)	<i>RSS</i> (-)	<i>RMSE</i> (%)
Pumpkin	0.9368±0.0096	412.1±1019.1	4.6469±0.3107	0.0853	0.5947±0.3148	3.8819
Pea	0.8444±0.0125	44.4995±11.3462	6.0215±0.1704	0.1323	0.4829±0.2837	3.6760
Sunflower	0.8456±0.1208	682.28±10.0564	5.6918±1.8082	0.2666	1.7852±1.3629	5.2700
Rice	0.7305±0.1331	7.8460±5.4342	7.4307±2.3167	0.4556	10.5576±1.3265	13.6481
Hemp	0.6378±0.1436	9.1463±5.1298	8.7523±2.6052	0.4478	8.7178±1.2054	13.8497

Source: results of own research.

The BET and GAB models and the monolayer concept on which they are based appear to be a useful tool in explaining various mechanisms of stability (Aviara, 2020). It is important to note that the product-specific relationship between water content and water activity is the basis for estimating the constants in these models (Condon, 2019). Even if these models fit to experimental data, this does not prove that they are of theoretical significance. This, in turn, supports the use of also empirical equations to model the sorption process (Labuza and Altunakar, 2020). Such equations can be used to study the diversity of sorption isotherms.

As long as the constants in the model have no physical meaning, any three-parameter model can effectively represent the sigmoid-shaped water vapor sorption isotherm. However, when constants are used to estimate the monolayer value, most empirical models are insufficient because this parameter does not exist. In such cases, it is safer to use, e.g., a four-parameter empirical model that does not require but also does not exclude the existence of a monolayer, for example the Peleg model (Pałacha and Sitkiewicz, 2010; Labuza and Altunakar, 2020).

This simplest four-parameter empirical model is applied to describe water sorption isotherms having both sigmoidal and non-sigmoidal shapes (Peleg, 1993). Its use allowed a perfect description of isotherms, as evidenced by the low *RSS* and *RMSE* values. The comparison of the parameters of the Peleg equation estimated for all the analyzed powders indicates that they differed significantly in their sorption properties (Table 6).

The specific sorption surface area of the tested powders was calculated based on the values of the monolayer estimated using the BET and GAB models. Moreover, the data estimated using the GAB model enabled determining the total volume of capillaries and the radius of capillaries filled as a result of initiating the capillary condensation phenomenon (Table 7). It should be noted, however, that the beginning of the condensation phenomenon was established based on a graphical analysis of the interpretation of sorption isotherms as the second inflection point.

The high-protein hemp powder had the largest specific sorption surface area. At the same time, it had a relatively low total capillary volume and a relatively large radius of capillaries that filled after capillary condensation initiation. These estimates allow speculating that the particles of this powder have a small number of capillaries with a fairly large radius (Condon, 2019). However, the specific sorption surface should be related not only to the physical specificity of the particles but also to their chemical properties, which determine the number and type of their hydrophilic groups (Pałacha and Sitkiewicz, 2010). The pumpkin seed powder was characterized by the smallest specific sorption surface area, with a relatively high total capillary volume and at the same time the smallest radius of capillaries that were filled after initiating capillary condensation. These estimates allow hypothesizing that the particles of this powder have a high number of very small capillaries (Condon, 2019). As a consequence, their specific surface area should be large. The specific sorption surface area is determined not only by the structure but also by the chemical composition

Table 6. Parameters of the Peleg equation for the analyzed protein powder samples

Protein source	Peleg's model parameters				Measures of fit	
	<i>A</i>	<i>B</i>	<i>D</i> (-)	<i>E</i>	<i>RSS</i>	<i>RMSE</i> (%)
Pumpkin	30.3964±2437.7	4.8076±26.0874	8.7375±2431.9	0.2438±101.67	0.1668±2.5923	1.8523
Pea	21.3841±2.0089	4.5188±0.6348	11.4676±0.8358	0.3391±0.0442	0.4212±0.3245	3.2057
Sunflower	27.7538±2.1907	5.9848±0.4532	11.1085±0.3523	0.2661±0.0209	0.1669±0.2043	1.9160
Rice	15.9344±1.4105	2.9191±0.7827	7.4459±2.2148	0.2736±0.1524	0.5983±0.3867	3.9286
Hemp	10.3324±0.4221	0.2684±0.0256	18.1209±0.9462	4.4193±0.3692	0.1017±0.1594	1.5093

Source: results of own research.

Table 7. Microstructural characteristics of the surface of the tested protein powder samples

Protein source	Specific sorption area (m ² g ⁻¹)		Total volume of capillaries (mm ³ 100 g ⁻¹ d.m.)	Capillary radius filled at <i>a_w</i> = 0.6 (g H ₂ O 100 g ⁻¹ d.m.)
	BET	GAB		
Pumpkin	169.0	163.3	58.66	1.28
Pea	198.7	211.6	60.61	1.45
Sunflower	206.1	279.7	57.54	1.48
Rice	150.8	261.1	55.25	1.38
Hemp	182.1	307.5	56.69	1.46

Source: results of own research.

(Table 1), which, in turn, affects the type and availability of hydrophilic groups. This statement is reinforced by taking into account the differences in the particle sizes of the tested powders (Table 2). A material with a high degree of fragmentation is characterized not only by a more developed surface than a material with a low degree of fragmentation but also by other interesting properties (Raval *et al.*, 2019). Meanwhile, the rice powder with the smallest diameters of its particles had a much smaller sorption surface area than the hemp seed powder having over 1.5 times larger particles.

The last aim of this study was to determine the relationship between the monolayer capacity (Tables 1, 2 and 5) and selected chemical and physical parameters of the powders along with selected statistics (Table 8). Due to the widely described advantages of the GAB model and the limitations of the BET model (Peleg, 1993; Lewicki, 1997; Basu *et al.*, 2006; Andrade *et al.*, 2011; Aviara, 2020), the monolayer capacity, estimated based on the GAB model, was used as a dependent variable in the multiple regression equations. The set of independent variables was limited to selected chemical parameters (protein, fiber, lipids) and selected physical parameters (diameter, circularity, elongation, and shape coefficient). The choice of chemical parameters was determined by their significant role in shaping sorption properties. In turn, the choice of physical parameters was determined by the results of the research presented in this paper, which showed that only the diameter, circularity, elongation, and shape coefficient differentiated the tested powders.

The high R^2 values (0.84–0.99), indicating a correlation between the dependent variable and the best combination of its predictors, showed that the parameters used to estimate the regression equations were well selected. However, based on the comparison of the R^2 values, it may be concluded that the monolayer capacity was best predicted by the regression equation with protein, fiber, and lipids (0.99) assumed as coefficients (predictors). The monolayer capacity was also predicted reasonably well by the regression equations with fiber, lipids, and circularity (0.97) and with fiber, protein, and diameter (0.97). The conclusion is that the sorption properties of the tested powder particles were strongly correlated with their chemical composition, with fiber present in each of the three cases considered. The significant role of fiber in shaping the sorption properties of the tested powders is important to emphasize, because it was not their quantitatively dominant component. Moreover, in the literature (Hébrard *et al.*, 2003), a view is presented that protein has five times higher sorption properties than carbohydrates, which include fiber. Therefore, it can be assumed that the role of fiber in shaping sorption properties should be considered separately from, for instance, the role of starch, which is usually a significant component of plant products.

Based on the comparison of the values of regression coefficients with their standard error values, it may be concluded that some coefficients attained the null value in many of the examined equations and, therefore, were not important elements of these equations. This statement in each case applies to equations in which one of the parameters of the regression equations were quantities describing such physical properties of the particles as elongation and the shape coefficient (Łomnicki, 2006).

The value of the standard estimation error (one measure of the accuracy of the regression equation) indicates the degree of dispersion of the residuals, *i.e.* the difference between the actual and predicted values. Small values (in most cases below 1) of this statistical parameter indicate that the predicted values are close to the actual values (Carlberg, 2012).

In addition, the F values determined for regression can be used to establish the probability of obtaining a high R^2 value by chance. The obtained values of the F statistics indicate that the estimates made are reliable (Carlberg, 2012). The regression equations relating the sorption properties of the tested powders to their chemical composition or chemical composition and diameter as well as circularity can be considered particularly useful in the context of the results reported by Murrieta-Pazos *et al.* (2014), Murphy *et al.* (2020), and Ding *et al.* (2020).

In summary, it may be concluded that the capacity of the monolayer, which is a parameter determining the sorption capacity of the tested high-protein powders of plant origin with different composition and physical parameters, is strongly related to their chemical composition. However, it is also likely that some physical parameters, especially those relating to particle size, may be factors that are important for this correlation.

4. CONCLUSIONS

The comparison of the parameters of the Brunauer, Emmet, and Teller; Guggenheim, Anderson, and de Boer; and Peleg models showed that the analyzed powders differed significantly in their sorption properties.

The Guggenheim, Anderson, and de Boer model best described the sorption properties of the powders, as determined by the root sum squared and root mean square error values and the wide range of data used to estimate the model parameters.

The sorption capacity of the high-protein plant powder preparations was related to their chemical composition and their particle size.

Fiber is particularly important in creating the sorption properties of plant powders, even those with very high protein content.

The diameter and circularity of particles are important physical parameters that determine the sorption properties of high-protein plant powders.

Table 8. Characteristics of multiple regression equations of the analyzed protein powder samples

Independent variables	Protein	Fiber	Lipids	Intercept term
Regression coefficient	0.1109	0.2949	-0.2273	-2.342
Standard error of regression coefficient	±0.0424	±0.0708	±0.0942	±4.567
R ²	0.9912	Standard estimation error	0.3004	Regression F value
Independent variables	Protein	Fiber	Diameter	Intercept term
Regression coefficient	0.1765	0.3998	-0.0928	-9.0341
Standard error of regression coefficient	±0.0438	±0.0818	±0.0884	±4.6701
R ²	0.9714	Standard estimation error	0.5415	Regression F value
Independent variables	Protein	Lipids	Diameter	Intercept term
Regression coefficient	-0.0616	-0.5215	-0.0337	16.4126
Standard error of regression coefficient	±0.0425	±0.2786	±0.2265	±5.1561
R ²	0.8416	Standard estimation error	1.2733	Regression F value
Independent variables	Fiber	Lipids	Diameter	Intercept term
Regression coefficient	0.1167	-0.4114	-0.0446	9.8465
Standard error of regression coefficient	±0.0447	±0.1571	±0.1430	±1.6074
R ²	0.9371	Standard estimation error	0.8026	Regression F value
Independent variables	Protein	Fiber	Shape coefficient	Intercept term
Regression coefficient	0.1976	0.4256	-0.8407	-11.5122
Standard error of regression coefficient	±0.0571	±0.1196	±6.0212	±7.8674
R ²	0.9410	Standard estimation error	0.7774	Regression F value
Independent variables	Protein	Lipids	Shape coefficient	Intercept term
Regression coefficient	-0.0530	-0.5115	-5.9084	19.4237
Standard error of regression coefficient	±0.0342	±0.1876	±7.1020	±5.4246
R ²	0.9043	Standard estimation error	0.9896	Regression F value
Independent variables	Fiber	Lipids	Shape coefficient	Intercept term
Regression coefficient	0.1024	-0.4265	-4.2468	12.5396
Standard error of regression coefficient	±0.0368	±0.0961	±4.5720	±3.3770
R ²	0.9629	Standard estimation error	0.6160	Regression F value
Independent variables	Protein	Fiber	Circularity	Intercept term
Regression coefficient	0.2002	0.4326	-0.0065	-12.3638
Standard error of regression coefficient	±0.0580	±0.1207	±7.0042	±8.7781
R ²	0.9398	Standard estimation error	0.7849	Regression F value
Independent variables	Protein	Lipids	Circularity	Intercept term
Regression coefficient	-0.0548	-0.5203	-7.5283	21.1059
Standard error of regression coefficient	±0.0305	±0.1683	±7.3400	±5.9083
R ²	0.9211	Standard estimation error	0.8987	Regression F value
Independent variables	Fiber	Lipids	Circularity	Intercept term
Regression coefficient	0.1033	-0.4291	-5.4363	13.6331
Standard error of regression coefficient	±0.0315	±0.0837	±4.5554	±3.5379
R ²	0.9715	Standard estimation error	0.5400	Regression F value
Independent variables	Protein	Fiber	Elongation	Intercept term
Regression coefficient	0.1976	0.4256	0.8407	-12.3529
Standard error of regression coefficient	±0.0570	±0.1196	±6.0212	±4.9108
R ²	0.9410	Standard estimation error	0.7774	Regression F value
Independent variables	Protein	Lipids	Elongation	Intercept term
Regression coefficient	-0.0530	-0.5115	5.9084	13.5153
Standard error of regression coefficient	±0.0342	±0.1876	±7.1020	±4.8269
R ²	0.9043	Standard estimation error	0.9896	Regression F value
Independent variables	Fiber	Lipids	Elongation	Intercept term
Regression coefficient	0.1024	-0.4265	4.2468	8.2927
Standard error of regression coefficient	±0.0368	±0.0961	±4.5720	±1.6463
R ²	0.9629	Standard estimation error	0.6160	Regression F value

Source: results of own research.

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